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Experiments on Vegetation Control with Native Pathogenic Fungi in the Southern Interior of British Columbia

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ABSTRACT

Fungi associated with diseases of forest weed species were tested for their mycoherbicide potential under greenhouse, growth chamber and field conditions. Several field treatments were applied, and subsequent changes to competing vegetation were measured in terms of foliar disease ratings and % cover estimates. Treatments which have shown positive responses are: hardwood overstory removal (resulting in mortality of aspen stumps); defoliation of thimbleberry (increased foliar disease rating in the subsequent year); foliar inoculation with *Septoria rubi* (increased disease rating of thimbleberry in the current year); 1.6 kg/ha glyphosate (some foliar damage to thimbleberry). Biological control using mycoherbicides is seen as a potential alternative to present methods, and also offers numerous ways of improving the efficacy and safety of conventional vegetation management strategies.

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INTRODUCTION

About 3 million ha of the more productive forest lands in British Columbia require some form of vegetation management to reach their potential within a reasonable time (Boateng 1986). Of these, roughly 1 million ha are not satisfactorily restocked after disturbance, have doubtful stocking, or are occupied by non-commercial brush species. There are serious obstacles to correcting this situation, particularly the backlog of brushed-in, unsatisfactorily restocked lands. Removal of brush by manual, mechanical or incendiary means is a temporary solution because of the rapid regrowth of brush species and invasion by seed-propagated species. Use of chemical herbicides has increased in volume, effectiveness and sophistication over the past two decades (Vanden Born and Malik 1984), but the use of chemicals in forestry is subject to increasing public opposition with the result that registration of new herbicides suited to forestry situations is unlikely. While there are many ways of tackling this immense backlog, the cost will still be high. Methods presently used are costly and sometimes ineffective. New approaches to vegetation management are needed.

One potential avenue now gaining widespread acceptance is biological control of unwanted vegetation. The classical approach to this, which involves introducing exotic pests to control weeds, has been practiced for decades (Harris 1986). However, since most forest weeds are native species which also have widespread amenity value, the classical approach generally should not be used. For the time being, biological weed control in forestry is best viewed as site-specific manipulation of indigenous pests and diseases to reduce the impact of competing vegetation. In North America, two of the earliest published accounts of potentially successful manipulations of indigenous diseases were by Wilson (1965) using the persimmon wilt fungus and by French and Schroeder (1969) using the oak wilt fungus. Although their ideas did not gain immediate acceptance in forestry, the concept grew in agricultural and aquatic ecosytems (Walker 1981; Charudattan and Walker 1982; Templeton 1982; Charudattan 1986; Mortensen 1986; Wymore *et al.* 1988) to the point that two mycoherbicides have been registered for commercial use in the U.S. and several others are in various stages of development (TeBeest and Templeton 1985). Activity in the field of mycoherbicides has grown exponentially in the 1980's.

In forest weed control, research on mycoherbicides is just beginning (Scheepens 1980; Wall 1977, 1983, 1986, 1990). Until recently, research on this aspect of pathology or weed management has not been a full-time effort at any institution. In 1986, however, a program was established at the Pacific Forestry Centre, focussing on mycoherbicide development for forest weeds.

For the project in the southern Interior, emphasis was placed on competing vegetation dominating backlog reforestation sites on potentially productive lands. This led to selection of cool, moist sites typified by the Interior Cedar - Hemlock biogeoclimatic zone and target species typical of these sites, such as aspen, paper birch, thimbleberry, raspberry, and fireweed (Angove and Bancroft 1983; Haeussler and Coates 1986). Diseases selected as sources of potential mycoherbicides were those found locally on forest weed species. A variety of foliar, stem and root rot diseases was selected. The expected roles of foliar diseases were twofold: sufficient defoliation to provide temporary release to suppressed conifers; and reduction in carbohydrate reserves, with subsequent weakening of the target plants' defenses against further stresses (Wargo 1981). Because a sufficiently virulent pathogen to achieve adequate defoliation could not be used at this early stage, the most common weed species, thimbleberry, was artificially defoliated in midsummer at about the stage of maximum leaf area, a stage of supposedly highest vulnerability to such a treatment (Coleman 1986). The stem diseases found were caused by wound pathogens (Boyce 1961; Coleman 1986), so their most logical application was to freshly cut stumps to prevent resprouting. Root disease fungi appeared initially to lend themselves to soil inoculation, possibly in conjunction with site preparation or planting.

Comparison of biological controls with contemporary vegetation management methods was one of the objectives of this study. At this stage of development of biological controls, this is a premature comparison, but including some of the major control methods, such as manual removal and the herbicide glyphosate, gave us benchmarks against which to measure efficacy. There was also an opportunity to combine biological with manual and chemical treatments. Stump treatments with a fungus to prevent

resprouting is an example of such a combination. Much research has been done on the effects of chemical herbicides on crop tolerance to disease (Altman and Rovira 1989). Low dosages of glyphosate are known to interfere with the defense mechanisms of plants (Levesque and Rahe 1989). Thus, glyphosate applied at sublethal dosages is considered a potentially good adjuvant for weak pathogens used as mycoherbicides.

The original objectives of this study were as follows: (1) to evaluate the relative efficacy of available biological controls for thimbleberry and associated hardwood species; (2) to measure the interactions among thimbleberry, overstory hardwoods, and conifer seedlings; (3) to evaluate the direct and indirect effects of selected biological control agents on conifers and other non-target vegetation; (4) to determine if these agents are viable options, by themselves or in conjunction with other control methods. This report discusses those objectives achieved to date.

MATERIALS AND METHODS

Collection and Culture of Pathogenic Fungi

Collection of diseased material began during the summer of 1987 (Table 1). To date, most collections have been made in the Castlegar, Salmo, Crawford Bay, Lardeau, Nakusp and Revelstoke areas. Collected materials were placed in polyethylene bags and kept out of direct sunlight during transportation to the Pacific Forestry Centre, where they were stored at 0°C. Within a week they were examined under a stereoscopic microscope and diseased tissues were selected for culturing and more detailed examination. To make cultures of diseased tissues, tissue surfaces were sterilized in a 10% solution of commercial sodium hypochlorite bleach for 1-3 minutes, and then small pieces placed on potato dextrose agar, malt agar, corn meal agar, or V8 juice agar (Dhingra and Sinclair 1985). Fungi growing from diseased tissues were subcultured and identified. Where fungus fruiting bodies were detected on diseased plant tissues, they were placed in a moist chamber to induce sporulation. Spores were then cast directly onto agar media or suspended in sterile distilled water and streaked onto the agar surface. Germinated spores were aseptically transferred to fresh agar media and resulting cultures identified.

TABLE 1. Fungi associated with diseases of non-commercial brush species in the southern interior of British Columbia

Host and fungus species	Disease symptoms
Alnus viridis ssp. sinuata, Sitka alder	
Chondrostereum purpureum (Pers. ex Fr.) Pouzar	Decay of cut stumps
Gnomonia sp.	Leaf spot
Haematostereum sp.	Decay of cut stumps
Betula papyrifera, birch	
Chondrostereum purpureum	Decay of cut stumps
Ceanothus sanguineus Pursh., buckbrush	
Cylindrosporium ceanothi Ell. & Ev.	Leaf spot
Populus balsamifera ssp. *trichocarpa*, black cottonwood	
Melampsora occidentalis Jacks.	Leaf rust
Septoria musiva Pk.	Leaf spot
Populus tremuloides, aspen	
Chondrostereum purpureum	Decay of cut stumps
Cytospora sp.	Stem canker
Graphium sp.	"Buboes canker" (Funk 1980)
Pollaccia radiosa (Lib.) Bald. & Clif.	Shoot blight
Ribes viscosissimum Pursh., sticky currant	
Cercospora sp.	Leaf spot
Rubus parviflorus, thimbleberry	
Alternaria sp.	Leaf spot
Botrytis sp.	Fruit rot, dieback
Cylindrocarpon destructans (Zinf.) Scholten	Root rot
Fusarium oxysporum Schlecht.	Root rot
Gnomonia sp.	Leaf spot
Hainesia sp.	Leaf spot
Naematoloma fasciculare (Huds. ex Fr.) Kar.	Root rot
Phragmidium occidentale Arth.	Leaf rust
Resinicium bicolor (Alb. & Schw. ex Fr.) Parm.	Root rot
Seimatosporium sp.	Leaf blotch
Septoria rubi West.	Leaf spot
Sphaerotheca sp.	Powdery mildew
Verticillium sp.	Root rot

Fungus cultures were grown on appropriate agar media in petri dishes at 20°C. When growth was complete, they were stored at 5°C.

Greenhouse and Growth Chamber Experiments

Propagation of test plants

Weed species used in greenhouse inoculation trials were collected as stem (*Populus balsamifera* L. ssp. *trichocarpa* (Torr. & Gray ex Hook.) Brayshaw) = black cottonwood), rhizome (*Rubus parviflorus* Nutt. = thimbleberry), or root (*Populus tremuloides* Michx. = aspen) cuttings in late autumn in the Nelson Region from apparently healthy trees or shrubs. Cuttings were stored at 0°C for 3 - 4 months and planted in a 50:50 mixture of sand and sphagnum peat containing 3 kg/m^3 of coarse dolomitic limestone, 2 kg/m^3 Osmocote 18-6-12, and 1 kg/m^3 of Micromax. Cuttings were kept in mist beds in a cool greenhouse until rooting had occurred and transplanted to plastic pots of 4.5 L capacity. During active growth, plants were top-dressed with Osmocote 18-6-12 (9-month release) at the rate of 14 g per pot.

In addition, thimbleberry plants were successfully grown from seed which had been stored for 2 years after collection in a peat-sand mixture at 0°C and from softwood cuttings (current year's shoots).

Foliar pathogenicity tests

Greenhouse and growth chamber pathogenicity tests are outlined in Tables 2, 3, 4, and 5. Spores of the test fungi were scraped from the surface of petri dish cultures and suspended in distilled water. If the fungus did not sporulate consistently, whole cultures were macerated in distilled water. Concentrations of propagules were adjusted to 1 million/mL and a wetting agent (0.1% gelatin or 0.05% Tween 20) was added before spraying. Fungal suspensions were applied to all foliar surfaces of the test plants with an atomizer. The plants were then usually placed in a Percival dew chamber set at an air temperature of 20°C for 24 - 48 hr before incubation in a greenhouse or growth chamber. As much as possible, greenhouse and growth chamber conditions simulated moderate summer conditions in British Columbia; 15-25°C with a 16-hr photoperiod. Plants were periodically observed for foliar disease symptoms and rated on a 0 to 5 scale (0 = no symptoms, 1 = scattered leaf spots, 2 = conspicuous leaf spotting, 3 = about 50% of leaf area diseased, 4 = 60-90% of leaf area lost to disease, 5 = plants dead).

To study the interaction between a chemical herbicide and a pathogen applied as a foliar spray, glyphosate (as the commercial formulations CLEARIT® 2 [CIL] or ROUNDUP® [Monsanto]) was prepared as 0.001 M or 0.002 M aqueous solutions (found by preliminary testing to cause no mortality), and applied to thimbleberry plants at the rate of 50 mL/m^2 (= approx. 0.08 or 0.16 kg/500 L per hectare). After 24 hr, plants were inoculated by being sprayed with the fungus, as above.

Root Inoculations

Autoclaved rhizome sections of thimbleberry, about 1 cm diam. x 5 cm long, were inoculated with the test fungus and incubated 6 months at 20°C. The fungus-colonized rhizome sections were then placed in the soil adjacent to the roots of the test plants, half of which were defoliated at the time of treatment. Alternatively, spore suspensions of test fungi were applied to the soil around the plant, or roots were dipped in spore suspensions and transplanted. Test plants were incubated in the greenhouse or shadehouse for a year, or until most plants had died, and periodically rated for disease by the same system used for foliar inoculations.

TABLE 2. Foliar inoculation trials under greenhouse and growth chamber conditions

Host and test fungus	Number of tests	Maximum disease rating[a]
Aspen		
Pollaccia radiosa	1	2
Black cottonwood		
Melampsora occidentalis	1	2
Thimbleberry		
Alternaria sp.	1	1
Botrytis sp.	1	2
Cylindrocarpon destructans	3	2
Fusarium oxysporum	1	3
Seimatosporium sp.	1	1
Septoria rubi	9	4
Verticillium sp.	3	3

[a] Rating system on 0 to 5 scale: = no symptoms; 1 = scattered leaf spots; 2 = conspicuous leaf spotting or other disease symptoms; 3 = about 50% of leaf area diseased; 4 = 60-90% of leaf area lost to disease; 5 = plants dead.

TABLE 3. Effects of temperature regime and dew treatment on foliar disease of thimbleberry inoculated with *Septoria rubi*

Day/night temperature cycle	Disease rating[a]	
	Dew period[b]	No dew period
18°/16°	3.6	3.8
20°/15°	3.0	—
25°/20°	2.4[c]	2.4[c]

[a] Disease rating on a 0 to 5 scale: 0 = healthy; 1 = scattered leaf spots; 2 = conspicuous leaf spotting; 3 = about 50% of leaf area lost to disease; 4 = 60-90% of leaf area lost, 5 = plants dead.

[b] Dew period of 24 hr in a Percival Dew Chamber.

[c] Very little *Septoria* leaf spot at high temperature regime. Damage mainly by powdery mildew.

TABLE 4. Effects of post-inoculation dew period and pretreatment with 0.002 M glyphosate on foliar disease of thimbleberry inoculated with *Septoria rubi*

Treatment	Disease rating[a]		
	6 weeks	8 weeks	11 weeks
Post-inoculation dew period[b]			
Septoria alone	1.3	1.7	2.3
Septoria + glyphosate	2.3	**3.3**	**4.3**
Control	1.0	1.3	2.7
No dew period			
Septoria alone	1.7	2.3	3.0
Glyphosate alone	2.0	**3.0**	**4.0**
Septoria + glyphosate	**2.3**	**3.3**	**4.3**
Control	1.0	1.3	2.0

[a] Disease ratings based on 0 - 5 scale: 0 = healthy; 1 = scattered leaf spots; 2 = conspicuous leaf spotting; 3 = about 50% of leaf area diseased; 4 = 60-90% of foliage blighted; 5 = plants dead. Bold-faced values significantly different from the control at $p = 0.05$.

[b] Plants placed in a Percival Dew Chamber for 24 hr.

TABLE 5. Interaction of 0.001 M glyphosate and foliar inoculation with *Cylindrocarpon destructans* on thimbleberry

Treatment	Disease rating[a]
Fungus only	2.8a
Fungus + glyphosate	4.3b
Glyphosate only	2.8c
Control	0.3d

a Disease ratings tallied 3 weeks after inoculation on a 0 to 5 scale: 0 = no disease; 1 = scattered leaf spots; 2 = conspicuous leaf spots; 3 = about 50% of leaf area lost to disease; 4 = 60-90% of leaf area lost; 5 = dead. Values followed by the same letter not significantly different at p = 0.05.

Field Tests

Selection of the study area

Only one study area could be satisfactorily managed during the initial stages of this work. It met a number of requirements: it has a backlog reforestation problem with a fairly uniform cover of weed species of widespread importance in the Interior; it is accessible by road during the growing season; it is in an isolated location, not usually visited by the general public; it is located on a potentially productive forest site; it is reasonably distant from streams in compliance with B.C. Ministry of Environment regulations.

The area chosen was a 31-ha clearcut at about 1000 m elevation, located in the Jordan Creek watershed near Genelle (map # 82F102, opening # 10). The area had been logged in 1981, and a 1984 regeneration survey had indicated inadequate conifer stocking. It was in the ICHa2 biogeoclimatic zone with an original cover of white pine (*Pinus monticola* Dougl.), lodgepole pine (*Pinus contorta* Dougl.), western redcedar (*Thuja plicata* Donn.), hemlock(*Tsuga heterophila* (Raf.) Sarg), larch (*Larix occidentalis* Nutt.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Cover in 1987 consisted of paper birch (*Betula papyrifera* Marsh.), trembling aspen, thimbleberry, and fireweed (*Epilobium angustifolium* L.) with scattered alder (*Alnus viridis* (Chaix.) DC. ssp. *sinuata* (Regel) Love and Love), buckbrush (*Ceanothus sanguineus* Pursh.), willow (*Salix* sp.), black cottonwood, raspberry (*Rubus idaeus* L.), bracken (*Pteridium aquilinum* (L.) Kuhn.), pearly everlasting (*Anaphalis margaritacea* (L.) C.B. Clark), and seedlings of white pine, redcedar, Douglas-fir, hemlock and larch. Dominant height of the brush cover in 1987 was about 3 m and the species distribution was fairly uniform considering the large number of skid trails and the sloping terrain.

Small-scale field trials

Small, closely spaced plots for preliminary evaluation of candidate pathogens and related treatments were established in the autumn of 1987. These were circular plots with a central stake arranged in five randomized, replicate rows of 20 plots for a total of 100. This allowed for a maximum of 20 treatments, which were applied to a 19.6 m^2 area (radius 2.5 m) around each stake. Treatments were applied in 1988 and the spring of 1989 (Table 6). Data on plot characteristics were recorded for a 4-m^2 area (radius 1.1 m) around the central stake. Initial plot data recorded were % cover estimates, number of stems, maximum heights of major species, and presence of less common species. In the spring of 1988, five healthy 1-0 Douglas-fir seedling plugs, size 415, were planted in each plot within 1.5 m of the plot centre, to provide a further measure of treatment efficacy (Tappeiner *et al.* 1987).

TABLE 6. Small-scale field tests applied to plots at Jordan Creek, 1988/89

Treatment	Overstory treatment	Understory treatment
A	Nil	Nil
B	Nil	*Septoria*, 30-7-88
C	Nil	Root fungi, 11-7-88
D	Nil	Defoliation + *Septoria*, 30-7-88
E	Nil	Defoliation + root fungi, 11-7-88
F	Removed, 11-7-88	Nil
G	Removed, 11-7-88	Defoliation, 11-7-88
H	Nil	Nil
I	Nil	*Septoria*, 15-6-89
J	Nil	Root fungi, 11-7-88
K	Nil	Defoliation, 11-7-88
L	Nil	Defoliation + root fungi, 11-7-88
M	Nil	Nil
N	Nil	*Septoria*, 22-7-89
O	Nil	Root fungi, 11-7-88
P	Nil	Defoliation, 11-7-88
R	Nil	Defoliation + root fungi, 11-7-88
S	Removed, 20-8-88	Nil
T	Removed, 28-10-88	Nil
U	Removed, 19-5-89	Nil

Intermediate-scale field trials

Fifty rectangular 50-m^2 plots, 5 x 10 m in dimension, were established in the spring of 1989. Areas selected had a cover of thimbleberry with a light overstory of hardwoods. Treatments are described in Table 7.

Foliar inoculations

Fungi used for foliar sprays in small and intermediate-scale field trials were grown on potato dextrose agar on petri dishes incubated at 20°C. When the dishes were covered with pure cultures of the fungus, they were transported to field quarters, where inoculum suspensions were prepared by the same methods used for greenhouse and growth chamber tests. Spore suspensions were adjusted to a concentration of 1 million spores per millilitre and gelatin solution added to make a final concentration of 0.1%. The suspensions were applied with a backpack sprayer at dusk on the same day at the rate of 5 L/100 m^2

Defoliation of thimbleberry

The effects of defoliation were tested on thimbleberry during midsummer (July 10-12, 1988) to simulate the effects of a serious foliar disease. Leaves were pulled from each shoot within each 19.6-m^2 test plot. During the same week, some plots were inoculated with root fungi. Two weeks later, when new leaves had regenerated, one set of defoliated plots were treated with a foliar fungus, *Septoria rubi* (Table 6).

Root inoculations

Autoclaved stem or rhizome sections of thimbleberry were inoculated with the test fungi and incubated at 20°C for 4 months. These were taken to the field and buried adjacent to the main lateral rhizomes of thimbleberry in 19.6-m^2 test plots (Table 6).

Crown removal and stump treatments

In this treatment, the aspen canker fungus *Cytospora chrysosperma* was applied to overstory hardwoods on 19.6-m^2 plots. The fungus was grown in flasks on sterile wheat bran at 20°C for 2 months. In the field, a slurry of the culture was prepared in SAE 30 motor oil and applied to the freshly cut stump surface. Detached crowns were left in the plot. Stumps were cut at a 1 m height so progressive deterioration or resprouting could be observed. This treatment was applied to five replicate plots in July, August and October 1988, and again in May 1989 (Table 6).

Manual brushing plus biologicals

This approach was tested in combination with two fungi, *Chondrostereum purpureum* and *Verticillium* sp., on intermediate-scale test plots in July of 1989. Brushing of 50-m2 plots was done with a scythe. Damage to conifer regeneration was avoided and the slash was left on the plots. Low shrubs such as thimbleberry were cut, as were overstory hardwoods. The macerated fungus cultures were applied in vegetable oil, using the same methods outlined under foliar sprays. Controls were brushed and treated with vegetable oil (Table 7).

TABLE 7. Intermediate-scale field trials established on 50-m^3 plots at Jordan Creek, 1989

Treatment number	Date	Treatment
I	19-5-89	Understory vegetation sprayed with *Cylindrocarpon destructans* spores.
II	19-5-89	Understory vegetation sprayed with spores of *Verticillium* sp.
III	19-5-89	Understory vegetation sprayed with *C. destructans* and *Verticillium* spores.
IV	19-5-89	Control; understory vegetation sprayed with distilled water.
V	20-7-89	Shrubs and hardwoods cut and sprayed with *Verticillium* sp. in vegetable oil.
VI	19-7-89	Shrubs and hardwoods cut and sprayed with *Chondrostereum purpureum* in vegetable oil.
VII	18-7-89	Control; shrubs and hardwoods cut and sprayed with vegetable oil.
VIII	22-7-89	Shrubs and frilled lower stems of hardwoods sprayed with 0.1 kg/ha glyphosate plus *C. destructans* spores.
IX	21-7-89	Shrubs and frilled lower stems of hardwoods sprayed with 0.1 kg/ha glyphosate.
X	21-7-89	Shrubs and frilled lower stems of hardwoods sprayed with 1.6 kg/ha glyphosate.

Chemical plus biological treatments

Glyphosate, in the commercial preparation Roundup®, was applied to 50-m^2 test plots at the rates of 1.6 and 0.1 kg/ha on July 22, 1989. Before application, overstory hardwoods were girdled at breast height and sprays were directed at the girdled stems and the understory vegetation. At dusk on the following day, half of the 10 plots treated with 0.1 kg/ha glyphosate were treated with a foliar spray of *Cylindrocarpon destructans* (Table 7).

Evaluation of results

To evaluate the small-scale field trials at monthly intervals during each growing season, maximum height was measured, and cover estimates and disease ratings were taken of thimbleberry, the main target species. Estimates of overstory hardwood and total understory shrub cover were also made periodically. Phenological records, such as leaf and flower development of thimbleberry and fall leaf senescence of major cover species, were also collected. During the dormant season, survival and heights of planted Douglas-fir seedlings were recorded.

In the intermediate-scale field trials, disease ratings of major species, including conifers, were recorded for each plot. Samples of injured or diseased tissues were examined for evidence of the test fungi and cultured on agar media.

To analyse data, treatments were compared using analysis of variance and Duncan's multiple range test. Where significant variation was observed, related treatments were lumped and compared to untreated controls using the "t" test. Ratings and percentages were transformed to arcsin % values before analyses.

RESULTS

Diseases and Damage Observed and Fungi Collected

Fungi observed on or cultured from diseased plant material in the southern Interior are listed in Table 1, along with hosts and disease symptoms. Besides the diseases listed, other conditions caused by insects or unknown factors were noted and collected. On thimbleberry, a stem gall caused by the insect *Diastrophus kinkaidii* Gillette was common and appeared to be potentially damaging (see Wangberg 1975). During the early summer of 1989, the rose curculio (*Rhynchites wickhamii*) caused considerable damage to thimbleberry foliage. A brown leaf spot of so-far undetermined etiology was common on thimbleberry in 1989. In all seasons, virus-like symptoms were noted on thimbleberry. Some virus diseases of this species have been described (Stace-Smith 1958; Credi *et al.* 1986; Stace-Smith and Shier 1988). On black cottonwood, the gall midge leaf spot described by Whitney and Baranyay (1968) was common in most seasons.

Not all fungal pathogens were considered as potential mycoherbicides. For instance, the rust and powdery mildew fungi are obligate parasites and very difficult to culture on artificial media. All collected specimens were filed in the Pacific Forestry Centre herbarium and cultures were maintained at 5°C in a special collection there.

Greenhouse and Growth Chamber Experiments

Foliar pathogenicity tests

A summary of foliar inoculation trials on thimbleberry and poplars is given in Table 2. It was found that one fungus, *Septoria rubi*, did not require a dew treatment for successful infection, but did require moderately low temperatures (15-20°) in the post-inoculation period (Tables 3 and 4). Also, *Cylindrocarpon destructans*, *Fusarium oxysporum*, and *Verticillium* sp., isolated from diseased roots and considered root pathogens, sometimes caused disease symptoms when applied as foliar sprays (Tables 2 and 5).

Thimbleberry treated with 0.002 M glyphosate (0.017 g/m^2) and inoculated with *S. rubi* on the following day became severely stunted and chlorotic and two out of six plants died within 3 months (Table 4). Thimbleberry treated with glyphosate alone became stunted and chlorotic but survived until the end of the experiment. Inoculation with *Septoria* alone resulted in severe necrotic spotting of leaves present at the time of inoculation, but healthy new leaves formed.

A combination of 0.001 M glyphosate and *Cylindrocarpon destructans*, applied as a foliar spray, was more effective than either alone (Table 5). Plants treated with *C. destructans* only developed leaf spots and foliar browning; glyphosate-treated plants became chlorotic with some necrotic foliage. When the fungus was applied together with the sublethal dosage of glyphosate, plants exhibited wilting, chlorosis and dieback, followed by development of sporodochia of *C. destructans* on the dead stems.

Root Inoculations

Potted thimbleberry plants, inoculated by infected rhizome segments buried in the soil adjacent to the roots, did not differ from the controls in foliage condition or rate of mortality. *Resinicium bicolor* and *Verticillium* sp. were tested.

Soil inoculations of thimbleberry with spore suspensions of *Cylindrocarpon destructans*, *Fusarium oxysporum*, or *Verticillium* sp. or the dipping of thimbleberry roots in spore suspensions of these fungi and then transplanting, did not result in any evidence of pathogenicity.

Small-scale Field Trials

Pretreatment variation in the study area

Analyses of variance of major species cover, heights, and numbers of stems taken before application of treatments indicated no significant differences. All plots but one had some thimbleberry cover and most had a hardwood overstory consisting largely of young birch and aspen. On a few plots, buckbrush or cedar formed the dominant cover.

Effects of foliar inoculations on thimbleberry

Inoculations with *Septoria rubi* caused a detectable increase in disease in two of four trials (Table 8). In 1988, inoculations of intact shrubs resulted in an increase during the following month in foliar disease rating. This effect did not carry over into the following year. Inoculation of the young foliage of previously defoliated shrubs had no measurable effect.

TABLE 8. Disease ratings of thimbleberry subjected to different treatments in small-scale field tests

Treatment	Disease rating[a]				
	Aug. 20 1988	Sept. 10 1988	July 20 1989	Aug. 21 1989	Sept. 21 1989
Defoliated, July/88	**0.8**	1.7	1.6	**2.3**	**2.8**
Root inoculation, July/88	1.9	2.7	1.1	1.8	2.2
Defoliated + root inoculation, July/88	**0.8**	**1.8**	1.3	1.9	2.2
Foliar inoculation, July/88	**2.8**	3.0	1.1	1.8	1.9
Defoliated + foliar inoculation, July/88	1.2	1.9	1.5	1.9	2.7
Foliar inoculation, June/89	2.1	3.3	**1.9**	**2.5**	**3.0**
Foliar inoculation, July/89	1.9	2.8	1.1	1.9	2.3
Overstory removed, July/88	1.9	3.0	1.1	**1.9**	2.3
Defoliated and overstory removed, July/88	1.1	**1.1**	1.7	2.3	**2.8**
Overstory removed, August/88	1.8	2.5	1.2	1.8	2.1
Overstory removed, October/88	1.8	3.0	1.2	1.9	2.6
Overstory removed, May/89	1.6	2.6	1.1	1.8	2.6
Untreated controls	1.8	2.8	1.3	1.6	2.1

[a] Boldfaced figures within a column significantly different from the untreated controls at $p = 0.05$.

In the following year, inoculations on June 15 resulted in a significant increase in disease for the duration of the growing season (Table 8). However, similar inoculations on July 22 had no detectable effect.

Effects of defoliation on thimbleberry

Removal of all leaves from thimbleberry in July 1988 had only a temporary effect on cover by that species. Within 6 weeks, there was no measurable difference in % cover between treated and control plots because of the rapid regrowth of new leaves. However, delayed effects appeared during the following summer in the increased incidence of necrotic leaf symptoms. The effects were not readily obvious, but various leaf spot diseases were noted and foliar disease ratings were higher than in untreated controls (Table 8).

Effects of root inoculations on thimbleberry

The root inoculations of July 1988 had no detectable effects, nor did defoliation plus root inoculation (Table 8).

Effects of overstory removal and stump treatment

In aspen there has been considerable stump dieback with little resprouting. Little dieback as a result of *Cytospora* treatment was noted on birch, willow or buckbrush.

Thimbleberry was associated with all types of overstory cover at the Jordan Creek site. It was generally denser (higher % cover, more stems per plot) in birch thickets where it was taller and had larger leaves than in more open areas. Overstory removal appeared to have no immediate measurable effect on thimbleberry.

Survival and growth of planted Douglas-fir

The Douglas-fir seedling plugs, averaging 25 cm in height at the time of planting, grew about 5 cm in the following year. Mean survival after 1 year was 94%. In plots with a dense hardwood overstory (>40% cover), mean survival and height was 86% and 25.2 cm, respectively. Where dense overstory cover had been removed during the summer of 1988, survival and height were 95% and 32.7 cm. The effects of other treatments were too small to evaluate over such a short time, when seedlings were essentially in the lag phase of growth.

Intermediate-scale Field Trials

Early spring inoculations

Thimbleberry and associated understory vegetation inoculated on May 19 with *Cylindrocarpon destructans* and *Verticillium* sp., alone and in combination (Table 7), have to date shown no symptoms of disease.

Glyphosate-*Cylindrocarpon* treatments

Thimbleberry in plots treated with 1.6 kg/ha glyphosate on July 21 (Table 7) showed some damage within a month. Other treatments have shown no effects to date.

Manual brushing plus pathogen treatments

Regrowth of thimbleberry and hardwoods from stump sprouts was evident within a month after treatment. Some of the aspen stump sprouts were infected by *Pollaccia radiosa*, but there was no evidence of the fungi used as inocula (*Chondrostereum purpureum* and *Verticillium* sp.) on the latest observation date.

For several weeks after treatment, conifers in these plots had an oily sheen as a result of treatment with vegetable oil.

DISCUSSION

Relative Efficacy of Biological Controls

It is too early to evaluate the full potential of biological controls and to compare the efficacy of the manipulations carried out in this study. Longer-term documentation will be necessary. Since biological control includes many possibilities, few of which have been tested or even considered, it should never be dismissed entirely.

Biological control may, in many cases, cause a slow decline rather than sudden mortality of target species. This could be an advantage in combating regrowth, which can result in relatively denser stands of competing vegetation within a few years after herbicide treatment or manual brushing. Where it is effective, biological control could have a more lasting effect with less regrowth of target weeds and less ingrowth of new competing species.

None of the pathogens selected for testing in this study were considered the best candidates for mycoherbicides. They were simply the organisms found in the environment in which they were to be tested and were known to be weak pathogens on the basis of the plant pathology literature. Preliminary experiments in the greenhouse and growth chamber could not adequately evaluate all of the variables which might affect the pathogens' efficacy as mycoherbicides. None of the effects observed in field tests could be considered adequate control, at least in the short term, but higher levels of infection could have resulted in herbicidal effects.

The manual defoliation treatments of thimbleberry at the time of maximum leaf development are an idealized effect of a foliar pathogen. An immediate but temporary effect would be partial release of suppressed conifers. A longer-term effect could be reduction in carbohydrate reserves and resultant slow decline (Wargo 1981; Coleman 1986). Some of the changes set in motion and associated with this type of decline are shifts in phenology and the resultant changes in susceptibility to frosts, insect attacks and viruses, the resultant reduction in the plants' ability to compartmentalize or otherwise resist infection by pathogens, and the resultant reduction in competitive ability.

Interactions among Target and Non-target Species

At the Jordan Creek site and during the stage of stand development studied, thimbleberry density was as great under hardwood cover as in openings. In fact, density was slightly higher in birch thickets than in more open locations. Growth habits differed: thimbleberry under hardwood cover had larger leaves and longer internodes than did open grown shrubs. Removal of overstory hardwoods had no immediate effects on thimbleberry.

Conifers, as expected, performed better under light cover than in thickets. The planted Douglas-fir, intended as a bioassay of treatment effects, had so far responded only to removal of dense overstory cover.

Effects on Non-target Vegetation

Few effects of treatments on non-target vegetation have been noted to date. Manual brushing and overstory removal undoubtedly caused some injury to conifers through wounding and burial (Holmsen and Whitehead 1988). Conifers treated with vegetable oil exhibited an oily sheen for several weeks after treatment, but it is not known if permanent injury occurred. Biological control should not appreciably affect the level of browse and cover for wildlife on a site since drastic reductions in cover are not anticipated. Because changes in cover are generally expected to be gradual rather than sudden, there is less likelihood of "shock" to tree seedlings or sudden ingrowth of non-target weeds.

A common concern about the use of plant pathogens as mycoherbicides is their possible non-target effect on beneficial forest plants, agricultural crops, and hardwoods scheduled for harvest. This fear could be largely overcome if it is remembered that these organisms are already in the environment, that they

are subject to natural controls, and that some form of stress treatment, such as wounding, as well as massive quantities of inoculum are usually necessary to make them effective as mycoherbicides. Because they are biodegradable and return to endemic levels after introduction to a site, they should pose little threat to the environment.

Potential of Biological Controls as Vegetation Management Tools

As mentioned above, biological controls cannot be dismissed entirely because they include innumerable possibilities, few of which will be tried. Few or none of the manipulations tried in this study may be developed into practical controls, yet they represent a promising beginning. They fall into two general categories: selective pathogens for specific target weeds; and the more general pathogens for mixtures of competing brush species.

The foliar pathogen studied in this project, *Septoria rubi*, showed some promise in greenhouse studies. Affected leaves were sufficiently diseased to become non-functional, and infection occurred without a dew period. Disadvantages were the difficulty in producing spores in culture and the failure of the fungus to behave systemically or to reinfect new foliage. To be effective as a mycoherbicide, a foliar pathogen would have to cause almost complete loss of foliage and prevent refoliation during the midsummer period. In greenhouse studies, *Septoria* caused partial defoliation and new, disease-free leaves developed. Use of low levels of glyphosate prevented refoliation. Leaf-spotting pathogens such as *Septoria* are attractive because of their host specificity and ease of application. However, they may not be suitable as mycoherbicides unless they can be induced to cause systemic infection or be combined with other pathogens or chemicals.

Since competing forest vegetation often consists of mixtures of species, it is possible that low grade, broad spectrum pathogens combined with other weed control tactics such as manual brushing or low levels of herbicides also have a place. In this study, *Cylindrocarpon destructans* plus low, normally sublethal dosages of glyphosate showed promise as a herbicidal preparation. *Chondrostereum purpureum*, which can invade hardwood stumps and prevent regrowth (Scheepens 1980; Wall 1990), may be a useful adjunct to conventional stand cleaning practices where regrowth is often a serious problem. *Cytospora chrysosperma*, tested in this project on hardwood stumps, may have a place in aspen management but it does not appear to affect other hardwoods.

The relative costs and convenience of using mycoherbicides have not yet been addressed. Generally, the organisms themselves and the materials used to culture them are inexpensive. A major factor to consider is the convenience, and hence the cost of application. The methods used in this study were far too cumbersome to be practical. Technology for formulation and application of biologicals is under development and will be applied to promising organisms in these studies.

Preconceived opinions on the mode of action of mycoherbicides can place limitations on procedures used in their development. Before this study, for instance, root inoculations were considered to be the appropriate method of applying root pathogens. This study indicates that foliar applications of some root pathogens may work much better. Also, a prolonged dew period was considered essential to the infection of plants by most foliar pathogens, but this study indicates that this is not the case for *Septoria rubi* on thimbleberry. These points show that, while the available literature and established concepts on plant disease are extremely valuable, prevailing theory must constantly be challenged. Furthermore, the present study has been based entirely on established chemical and manual methods of vegetation control. Departure from these could open valuable new approaches.

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